# DEVELOPMENT OF A PROCESS FOR DETECTING NONTHERMAL EFFECTS OF MICROWAVE ENERGY ON MICROORGANISMS AT LOW TEMPERATURE[1]

MICHAEL KOZEMPEL[2], RICHARD D. COOK, O. JOSEPH SCULLEN and BASSAM A. ANNOUS

*U.S. Department of Agriculture*
*Agricultural Research Service*
*Eastern Regional Research Center*
*600 East Mermaid Lane*
*Wyndmoor, PA 19038*

Accepted for Publication November 9, 1999

## ABSTRACT

*We developed an experimental process capable of isolating thermal and nonthermal effects of microwave energy relative to the destruction of microorganisms at low temperature. The concept combines instantaneous energy input to the food system by microwaves with rapid removal of thermal energy. The process used a double tube heat exchanger inside a continuous microwave dryer. The outer tube was transparent to microwaves, whereas the inner tube was stainless steel and was used for cooling the system. The microwave energy, 5-6 kW power, was absorbed by the process fluid in the annulus. The cooling water flowing in the inner tube removed the thermal energy from the process fluid to control temperature at or below 45C. The process was at turbulent flow to assure a uniform temperature and dwell time. There were no detected nonthermal effects from microwave energy for yeast,* Pediococcus *sp.,* Escherichia coli, Listeria innocua, *or* Enterobacter aerogenes *in various test fluids, such as water, liquid egg, beer, apple juice, apple cider, and tomato juice.*

## INTRODUCTION

Most foods interact with electromagnetic fields, absorbing energy and

[1]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

[2]Author for correspondence. Tel: 215-233-6588; Fax: 215-233-6795;
E-mail: mkozempel @ arserrc . gov

generating heat. The most common electromagnetic fields employed in the food industry are microwaves at 915 and 2450 MHz and radio frequency at a nominal 27 MHz.

There has been an ongoing debate for over fifty years whether there are nonthermal effects associated with electromagnetic energy. Just a few of the authors reporting results which appeared to indicate nonthermal effects are Culkin and Fung 1975; Webb and Dodds 1968,1969; and Olsen 1965.

However, for every author reporting an apparent nonthermal effect there is another author reporting no such thing, such as Carroll and Lopez 1969; Goldblith 1966; Goldblith and Wang 1967; Tong 1996; and Brown and Morrison 1953. Mertens and Knorr (1992) published an excellent review of nonthermal processes for food preservation. After presenting both sides of the debate, they concluded "whether or not these phenomena are real and can be applied in the food industry remains questionable and still needs to be demonstrated".

There is also the possibility that electromagnetic energy may act in a synergistic way to magnify the thermal effect. Ramaswamy and Tajchakavit (1993) reported microwave energy was substantially more effective than conventional thermal energy for pectin methyl esterase inactivation indicating the possibility of some complementary nonthermal effects. Reznik and Knipper (1994) reported "liquid egg pasteurized with a combination plate and frame heat exchanger followed by an RF electric heater exhibits several distinct advantages over liquid egg which was conventionally pasteurized. There is a higher degree of microbial kill. There is less regrowth of bacteria even when the egg is maintained at room temperature. In the refrigerated liquid egg, bacteria counts actually decreased for a few days after processing".

Several theories have been advanced to explain how electromagnetic energy might kill microorganisms. These theories are summarized in a review by Knorr *et al.* (1994). There is the "dielectric rupture theory" of Zimmermann *et al.* (1974) in which an external electric field induces an additional transmembrane electrical potential which is larger then the normal potential of the cell. At sufficient potential the cell membrane ruptures, resulting in pore formation, increased permeability, and lost cell integrity.

Kinosita and Tsong (1977) presented a mechanism for pore development caused by pulsating electric fields. They present evidence of migration of species across the cell membrane and resultant destruction of the cell.

Mertens and Knorr (1992) discuss the use of oscillating magnetic fields disclosed in a world patent (Anon. 1985). "The patent suggests that the oscillating magnetic field couples energy into the megneto-active parts of large biological molecules with several oscillations. When a large number of magnetic dipoles are present in one molecule, enough energy can be transferred to the molecule to break a covalent bond. It is assumed that certain critical molecules in a microorganism, like DNA, or proteins, could be broken by the treatment, hence destroying the

microorganisms or at least rendering it reproductively inactive".

There is the theory of selective energy absorption or differential heating in which the bacteria selectively absorb the electromagnetic energy. Although unlikely in a liquid system, there is precedence for such a phenomena on a macro scale in the response of insects in stored grain. Nelson and Charity (1972) found the degree of selective heating depends upon the relative values of the dielectric properties. They found differences in the loss factor between insects and grain.

Nonthermal effects due to electromagnetic energy is a controversial topic because of the difficulty in proving or disproving the phenomena. Frequently, experimental results are presented as a comparison to expected results based on *D* values. However, this is unreliable since "many parameters besides temperature.... control the stability of a biological.... system. Control experiments have to be conducted before any conclusions can be reached" (Tong 1996). Even the growth medium affects the stability of microorganisms (Annous and Kozempel 1998). A definitive experiment requires the isolation of the thermal effect from the nonthermal effect. A definitive process is probably impossible short of experimentally and nonintrusively measuring the temperature of each particle to test for selective heating. However, a very close approximation can be achieved by applying microwave or RF energy to a continuous steady state process while simultaneously removing the thermal energy to maintain a low temperature. A definitive continuous process must have turbulent flow to maintain a uniform temperature and time distribution. To establish nonthermal effects it is necessary to compare directly the process with microwave or RF energy input to a purely thermal process at the same temperature and in the same experiment, e.g. same bacteria, same flow rate, essentially all variables identical.

In a preliminary study Kozempel *et al.* ( 1997) reported on a pilot plant, batch, full recycle process using microwave energy simultaneous with cooling so temperatures remained below 40C. Sufficient energy was introduced in the microwaves chamber to reach the normal pasteurization temperature. Simultaneous cooling maintained the temperature below 40C. The process, by its very design, was not definitive. There was recontamination of the test liquid during recycle. Flow within the microwave chamber was not turbulent. Datta and Liu (1992) teach that destruction of microorganisms is associated with food elements as opposed to physical locations. Food elements are not fixed during processing. Ideally, every liquid element must be followed or accounted for. The temperature-time history is needed for every liquid element. The temperature-time distribution within the fluid in this system was unknown. Initial results appeared to indicate nonthermal kill for several fluids such as water, 10% glucose solution, and apple juice. If true, they offered the exciting possibility of developing a nonthermal process.

In a modification of this process (Kozempel *et al.* 1998), we used two alternate feed tanks with one tank acting as a receiver. Recycle alternated between the two feed tanks so recontamination of product with feed was minimized. However, it

was still not practical to achieve turbulent flow within the microwave chamber, which meant the dwell time was not uniform and there was an undefined temperature distribution. The data suggested there was bacterial kills in some fluids with microwave energy at low temperatures. But, this process was still more speculative than definitive.

The objective of this research was to use the knowledge gained from the previous two experimental processes to develop a definitive process capable of detecting nonthermal microwave killing of microorganisms.

## MATERIALS AND METHODS

The experimental system is a modification of our batch processes previously reported (Kozempel *et al.* 1997, 1998). The concept combines instantaneous energy input to the food system by microwaves with rapid removal of thermal energy. We used a double tube heat exchanger inside a continuous microwave dryer. The outer tube was polypropylene which is transparent to microwaves, whereas the inner tube was stainless steel through which tap water flowed to control the temperature. Process fluid circulated in the annulus counter-current to the cooling water. The microwave energy was absorbed by the process fluid in the annulus but was reflected by the inner tube. The cooling water flowing in the inner tube removed the thermal energy from the process fluid to control the temperature.

Figure 1 presents the experimental process, III. There was a single 190 L stainless steel feed tank. (There was no recycle of product as was done in the preliminary studies). A sanitary lobe pump, TriClover rotary pump, Kenosha, WI, model # PRED3-lM-UC6-ST-S, pumped the process fluid through a 7 kW 2450 MHz continuous microwave dryer, Cober Electronics Inc., Stamford, CT, at a controlled flow rate of 0.35 to 0.50 kg/min. The double pipe heat exchanger was inside the microwave dryer. The outer pipe of the heat exchanger, which contained the process fluid, was polypropylene, Sanitech -T 1-1/2 in. sanitary pipe, 33.8 mm inner diameter. The inner pipe was stainless steel, 25.4 mm outer diameter. To achieve turbulent flow while maintaining sufficient dwell time in the microwave chamber, the double pipe heat exchanger was assembled as an elongated loop with a second sanitary lobe pump, TriClover rotary pump, Kenosha, Wisconsin, model PR25-1½ M - ST - S. The second lobe pump circulated the fluid within the microwave chamber and the turns at the ends at 45 L/min, sufficient to achieve turbulent flow. Each leg of the double pipe heat exchanger had a separate cooling tube with cool tap water flowing counter current to the process fluid.

Process fluid exited the process in direct proportion to the inlet rate established by the first pump. The system can be visualized as depicted in the upper center of Fig. 1. Visualize the microwave section as a continuous stirred tank which receives the microwave energy. The feed stream enters with the bacteria concentration of

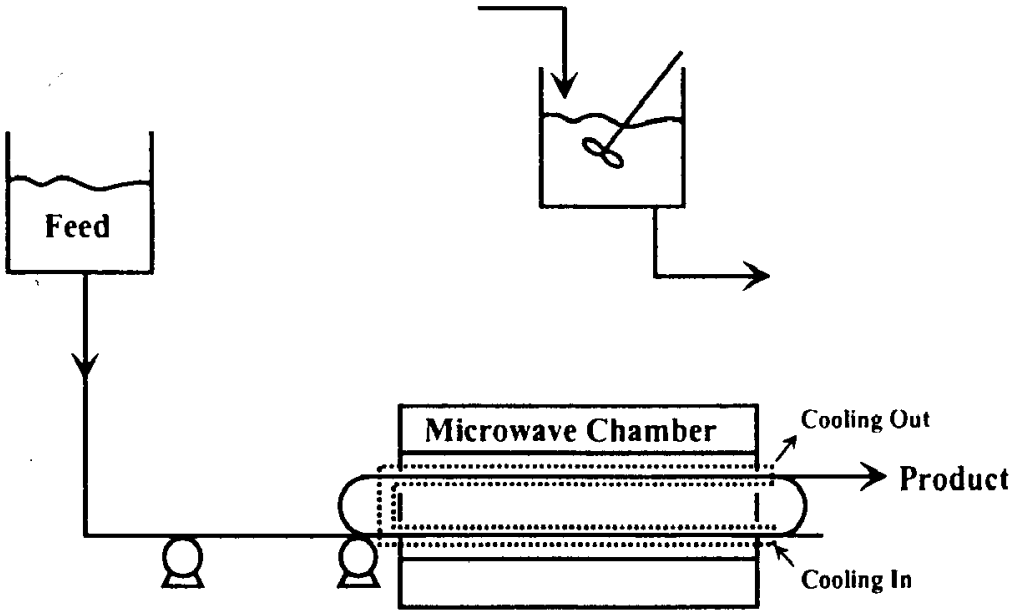


FIG. 1. PROCESS III FLOW SHEET WITH A WELL MIXED MICROWAVE TREATMENT SECTION AND NO AFTERCOOLER

the raw product, is treated in the microwave continuous stirred tank, and leaves as the product stream with the same concentration as the continuous stirred tank.

**System Preparation**

The system was sanitized before introducing the test fluid and bacteria by circulating hot water (>65C) through the system. Following sanitation of the process equipment, 57 or 114 L of feed (depending on the experiment) were charged to the feed tank and inoculated with the test microorganism. The test microorganisms were: *Pediococcus* sp. NRRL B-2354 supplied by L.K. Nakamura (U.S. Department of Agriculture, Peoria, IL), *Escherichia coli* K-12 and *Listeria innocua* SA3-VT supplied by P.M. Fratamico (U.S. Department of Agriculture, Wyndmoor, PA), and *Enterobacter aerogenes* B-199 obtained from Vivolac Cultures Corp. (Indianapolis, IN). All cultures were maintained on tryptose agar plates at 4C (TA; Difco Laboratories, Detroit, MI) with biweekly transfers to maintain strain viability. Unless otherwise indicated, the growth temperature for *Pediococcus* sp. was 28C, and for *E. coli*, *L. innoucua*, and *E. aerogenes* was 37C. *Pediococcus* sp., *E. coli*, and *E. aerogenes* were grown in tryptone glucose yeast extract (TGY) broth which was formulated in our laboratory (tryptone, 5 g; yeast extract, 5 g; glucose, 1 g; potassium phosphate dibasic, 1 g; double-distilled deionized water, 1 L; pH 7.00). Brain heart infusion (BHI; Difco) prepared in distilled water according to the manufacturer's guidelines was used to grow

*L. innocua*. A late exponential phase culture grown in the appropriate medium was used at 1% (vol/vol) level to inoculate 500 mL of the same medium. Cultures were grown to stationary phase, harvested by centrifugation for 10 min at 4C, washed once with sterile distilled water, and suspended in the experimental menstruum to a final concentration of ca. 6.5 log cfu/mL.

After inoculation, the feed was pumped through the equipment displacing the sanitizing water. To confirm that no extraneous cause was responsible for killing the bacteria, the fluid was processed with the microwave generator off for 1 h, and the bacterial concentration in the feed tank and the product stream monitored to be sure there was no change.

Microwave power was applied at 5-6 Kw and the temperature recorded continuously at both ends of the microwave chamber.

### Sampling and Analysis

Samples were collected in triplicate from the product line and the feed tank, and were serially diluted in 0.1% peptone (Difco) and surface plated on TA plates using the spiral plating system, Autoplate 4000 (Spiral Systems Instruments, Inc., Bethesda, MD). The plates were then inoculated at 37C for 18-24 h, and the survivors were enumerated using a laser bacterial colony counter, model 500A (Spiral System Instruments, Inc.).

Most of the test fluids were purchased from local supermarkets. Beer was prepared in house from commercially available beer brewing kits. Apple juice and cider were both purchased from supermarkets and from a local producer. The malic acid for making synthetic apple juice was supplied by the local apple cider producer. Liquid egg products were purchased from a local processor.

## RESULTS AND DISCUSSION

### Background

Our previous research results (Kozempel *et al.* 1997, 1998) using the earlier two processes which included product recycle and nonuniform temperature and time distribution gave tantalizing indications of kill of microorganisms in some liquids with microwave energy at low temperatures. The responses of the processes depended on the particular bacteria/food combination. The data suggested, but was not conclusive, that microwave energy could kill bacteria without heat. Yeast was reduced by 2.5 log and *Pediococcus* sp. by about 1 log in beer (Kozempel *et al.* 1998). There was a good kill in apple juice, ~5 log, when the *Pediococcus* sp. was grown in TGY but almost no response when TSB media was used. The process had no effect on *Pediococcus* sp. in skim milk and only a slight reduction in bacteria

in tomato juice (~0.5 log) and pineapple juice (~1.5 log). These data were more exploratory than definitive.

**Process**

The previous processes were not expected to be feasible processes, but only intended to test the possibility of destroying bacteria at low temperatures, to justify further research, and to lead to development of a near definitive process to detect the existence of nonthermal microwave destruction of microorganisms. The two previous processes had two known major experimental problems. The product was recontaminated when recycled and the flow rate was low (not turbulent) requiring knowledge of the temperature-time history of each element of liquid. Process III, Fig. 1 eliminated recycle and temperature-time history problems by use of turbulent flow through the microwave chamber. The second pump, the circulating pump, ran at 45 L/min, to achieve a sufficient Reynolds number for turbulent flow. Under turbulent flow conditions, the liquid can be considered well mixed with nearly uniform temperature-time history. To achieve a sufficient dwell time in the microwave chamber, the piping formed a loop in the microwave chamber for recycling the fluid at high flow rate; thus simulating a continuous stirred tank. The actual dwell time in the process was determined by the flow rate of the first pump. The second pump merely produced turbulent flow. In this system, there was no recycle to the feed tank.

There are two major advantages to this process. Microwave systems have hot spots, which result in heterogeneous over or under heating. The high recycle and turbulent flow in the microwave chamber essentially distributed uniformly the effect of exposure of the fluid to hot spots. As a result the fluid was uniformly heated only for the specified time.

In conventional pasteurization systems, the process fluid first gets heated to the pasteurization temperature and then held for the prescribed time. A second advantage to this process is the incoming fluid almost instantly reaches the temperature of the fluid in the microwave chamber. There is virtually no heat up time.

**Experimental Findings**

The process was used to detect any nonthermal effects due to microwave energy at low temperatures on various fluids and microorganisms, Table 1. Table 1 lists the systems studied and the mean bacteria counts for the feed and product samples. The feed and product means were compared statistically when more than one triplicate of product samples was taken. No system was statistically significant ($p \leq 0.05$). *Pediococcus* sp., *E. coli*, or *L. innocua* in water exhibited no effect. There was no effect on yeast in beer. Liquid egg white inoculated with *Pediococcus* sp. showed no bacteria destruction. Microwave energy had no effect on liquid whole

TABLE 1.
SYSTEMS EXHIBITING NO REDUCTION IN MICROORGANISMS

| Test Fluid | Microorganism | Temperature, C | Dwell Time, min (microwave chamber) | Feed | | | Product | | | Thermal | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean[1], log cfu/ml | (n) | SD | Mean[1], log cfu/ml | (n) | SD | Temp., C | Log cfu/ml |
| water | *Pediococcus* sp. | 26 | 3.4 | 6.84 | (3) | 0.017 | 6.94 | (3) | 0.026 | | |
| water | *Pediococcus* sp. | 40 | 7.2 | 6.05 | (4) | 0.033 | 6.13 | (6) | 0.045 | | |
| water | *Pediococcus* sp. | 41 | 7.3 | 5.53 | (2) | 0.014 | 5.51 | (4) | 0.054 | | |
| water | *E. coli* | 36 | 7.3 | 6.76 | (1) | | 6.81 | (2) | | 62.2 | <1.32* |
| water | *E. coli* | 41 | 9.7 | 5.64 | (2) | | 5.64 | (2) | 0.007 | 60.0 | 3.38* 2.96* |
| water | *L. innocua* | 44 | 3.6 | 5.98 | (1) | | 6.06 | (1) | | | |
| beer | yeast | 39 | 7.0 | 5.51 | (3) | 0.095 | 5.63 | (6) | 0.067 | | |
| beer | yeast | 40 | 7.4 | 6.47 | (2) | 0.042 | 6.48 | (3) | 0.035 | | |
| egg white | *Pediococcus* sp. | 45 | 2.7 | 6.44 | (2) | 0.382 | 6.31 | (2) | 0.148 | 58.3 | 3.90* |
| whole egg | *Pediococcus* sp. | 41 | 6.0 | 6.64 | (2) | 0.021 | 6.65 | (3) | 0.010 | 57.9 | 3.80* |
| whole egg | *Pediococcus* sp. | 45 | 2.7 | 6.78 | (2) | 0.007 | 6.71 | (1) | | | |
| whole egg | *E. coli* | 45 | 2.9 | 6.99 | (2) | 0.099 | 7.01 | (1) | | 56.9 | 4.97* |
| whole egg | *L. innocua* | 45 | 2.5 | 7.47 | (1) | | 7.53 | (1) | | | |
| whole egg | *E. aerogenes* | 42 | 3.2 | 6.75 | (3) | 0.095 | 6.83 | (1) | | 58.3 | <1.32* |
| whole egg | *E. aerogenes* | 15 | 3.2 | 6.98 | (2) | 0.099 | 6.79 | (1) | | | |
| tomato juice | *Pediococcus* sp. | 29 | 2.0 | 6.14 | (4) | 0.030 | 6.07 | (5) | 0.040 | | |

(n) – number of means
1 – Mean of samples taken in triplicate and analyzed in duplicate
*- Statistically significant difference from the product mean at $p \leq 0.05$.

egg inoculated with *Pediococcus* sp., *E. coli*, *L. innocua*, or *E. aerogenes*. *Pediococcus* sp., was unaffected when inoculated in tomato juice. These results indicate microwave energy at low temperature has no effect on microorganisms, at least in these systems. The obvious conclusion is that there is no nonthermal effect on microorganisms in these systems associated with microwave energy.

Including startup and cleanup, most experiments encompassed an entire working day. However, in the experiments when there was sufficient time, the cooling water was turned off to observe the thermal effect. These data are included in Table 1. In all cases, there was a statistically significant ($p \leq 0.05$) difference between the product mean and the thermal mean (kill).

Figure 2 shows there was about a 2 log kill of *Pediococcus* sp. in prune juice. When switched to pineapple juice the kill was less than 1 log. Apple juice presented interesting results, Fig. 3. The bacteria count in the feed tank dropped during the experiment. Something in apple juice kills *Pediococcus* sp. The product stream had a lower count than the feed tank by about 2 log for the first h with the microwave unit on. At 1 h, the microwave unit was turned off. During this second h, the count in the product rose and became equal to the feed tank. The cycle was repeated with the same response in the next 2 h. Although the count dropped in the feed tank, there clearly was killing associated with the microwave energy. Figure 4 shows similar results due to microwave energy for apple cider; although, the magnitude of kill was far less than in apple juice. Note that the bacteria was stable in the feed tank. Whatever kills the *Pediococcus* sp. in apple juice has little or no effect in cider.

This creates the dilemma of explaining the apparent kill in the earlier research and not in Process III. One possibility might be the presence of laminar flow/hot spots in process II versus turbulent flow in process III. To test this possibility, the circulating pump in process III was completely stopped to eliminate turbulent flow and create a once through system (one pass) as in process II. With a dwell time of 3.5 min in the microwave chamber, there was no kill of bacteria in water or yeast in beer. Laminar flow/hot spots versus turbulent flow does not appear to explain the contradiction.

Another possibility is insufficient dwell time in the microwave chamber. To test this possibility, water was inoculated with *Pediococcus* sp., the circulating pump was turned on and the feed pump turned off (no throughput). It was essentially a well mixed batch system. At half hour intervals the feed pump was run for 2 min to take a sample. After 1.5 h exposure to microwave energy at low temperature, there was no kill. Dwell time doesn't account for the kill in the earlier microwave system studies either.

Another possibility is a synergistic effect of the microwave energy and something in the liquid which injures or kills the microorganisms. For example, some property of apple juice might injure or kill *Pediococcus* sp. The obvious suspect is pH. Table 2 shows the effect of products of various pH on *Pediococcus* sp.

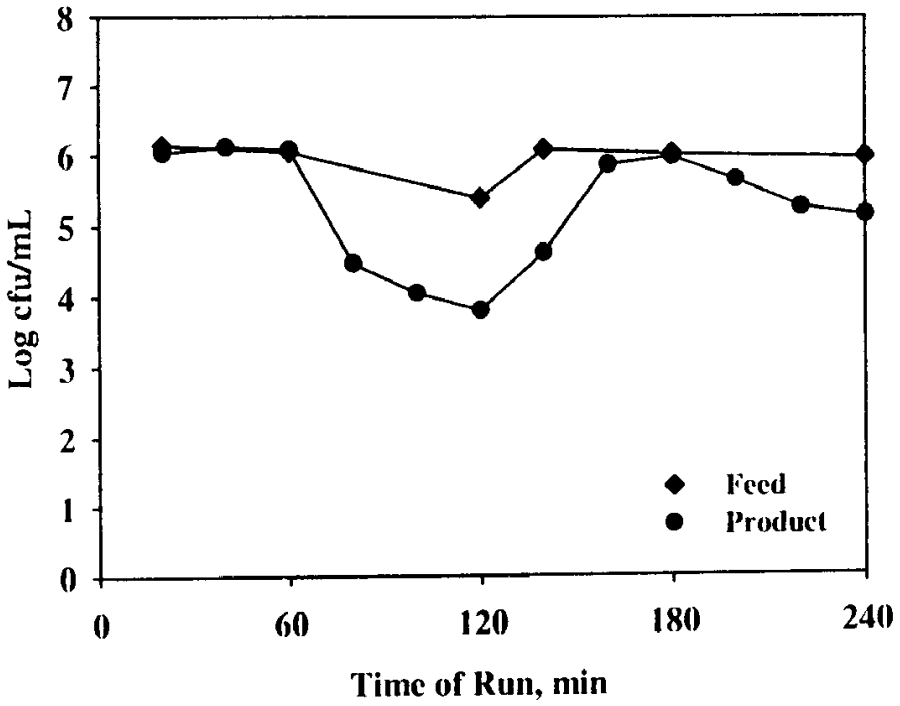


FIG. 2. SUBLETHAL MICROWAVE PROCESS III RESULTS WITH *PEDIOCOCCUS* SP. IN PRUNE JUICE AND PINEAPPLE JUICE

◆Feed; ●Product. Microwave unit turned on at 60 min; off at 120 min; on at 180 min. Switch from prune juice to pineapple juice at 120 min.

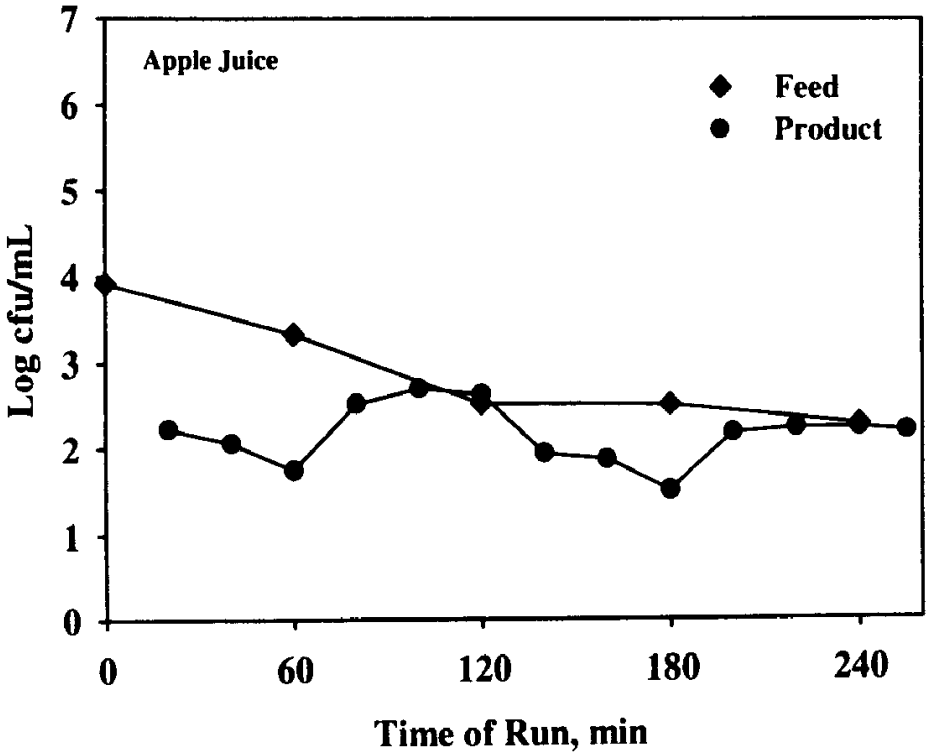


FIG. 3. SUBLETHAL MICROWAVE PROCESS III RESULTS WITH *PEDIOCOCCUS* SP. IN APPLE JUICE

◆Feed; ●Product. Microwave unit turned on at 0 min; off at 60 min; on at 120 min; off at 180 min.

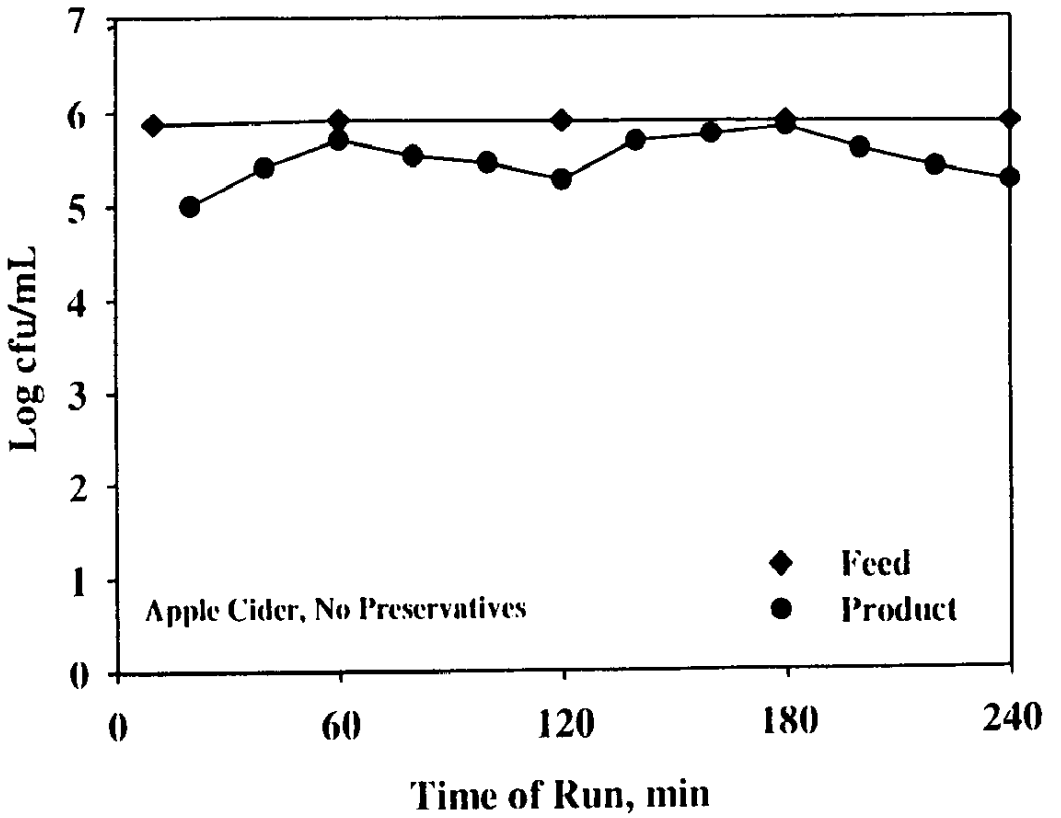


FIG. 4. SUBLETHAL MICROWAVE PROCESS III RESULTS WITH *PEDIOCOCCUS* SP. IN APPLE CIDER (NO PRESERVATIVES)
◆Feed; ●Product. Microwave unit turned on at 60 min: off at 120 min; on at 180 min.

TABLE 2.
SURVIVAL OF *PEDIOCOCCUS* SP. IN VARIOUS LIQUID FOODS, LOG CFU/ML

| | Time After Inoculation, h | | |
|---|---|---|---|
| Product (pH) | 0 | 5 | 22 |
| Water | 6.89 | 6.83 | 6.81 |
| Pineapple juice (3.7) | 6.75 | 6.64 | 3.84 |
| Prune juice (4.2) | 6.91 | 6.78 | 3.63 |
| Tomato juice (4.4) | 6.90 | 6.77 | 6.44 |
| Skim milk (6.9) | 6.93 | 6.76 | 6.63 |
| Apple cider (3.9) | 6.93 | 6.74 | 5.59 |
| Clear apple juice (4.0) | 6.32 | 5.98 | 5.59 |
| Orange juice (3.9) | 6.88 | 6.44 | 5.19 |
| Beer (4.0) | 6.93 | 6.31 | 4.79 |
| Cloudy apple juice (3.9) | 6.76 | 5.78 | 4.75 |
| Grapefruit juice (3.5) | 6.84 | 5.67 | 3.36 |
| Grape juice (3.6) | 6.23 | not detected | ----- |

Surprisingly, pH is not a factor. In 5 h the bacteria level dropped for apple juice (pH 3.9-4.0), grapefruit juice (pH 3.5) and disappeared for grape juice (pH 3.6). In the same pH range, pineapple juice (3.7), apple cider (3.9), and orange juice (3.9) were stable. *Pediococcus* sp. is stable in water, Table 2.

Figure 5 shows *Pediococcus* sp. in water was not affected by the microwave energy. In one run, we ran water for 2 h and switched to a synthetic apple juice (water, glucose, and malic acid) at 2 h. With the microwave unit off, there was no change in bacteria level - no kill. At 3 h, the microwave unit was turned on. There was an abrupt drop in bacteria in the exit stream. The suspected cause was a combined effect of microwave heating and malic acid.

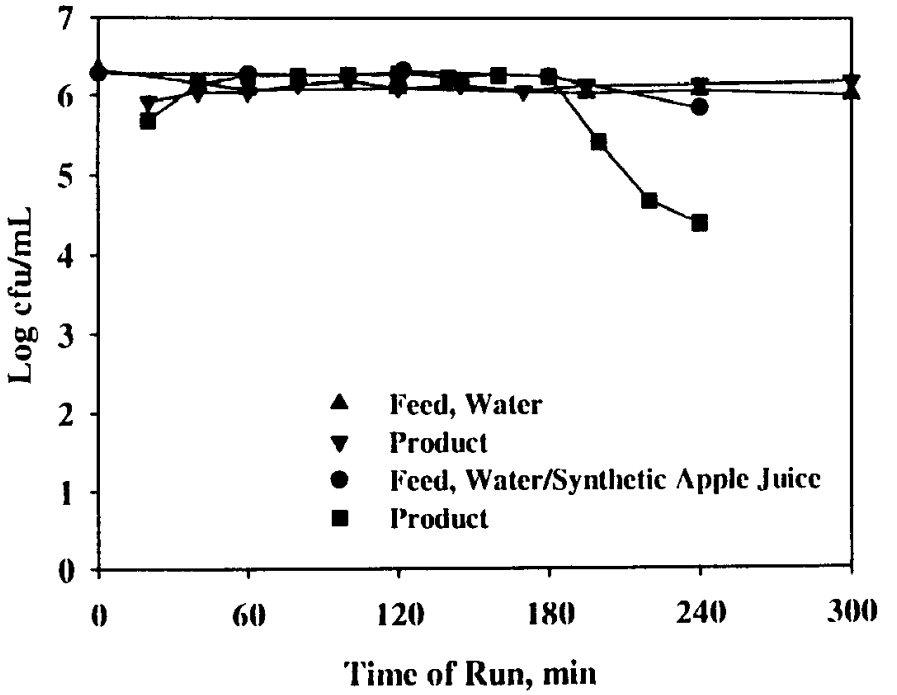


FIG. 5. SUBLETHAL MICROWAVE PROCESS III RESULTS WITH *PEDIOCOCCUS* SP. IN DEIONIZED WATER AND WITH SYNTHETIC APPLE JUICE (5.2% GLUCOSE, 0.19% MALIC ACID)

Microwave unit turned on at 60 min; off at 120 min; on at 180 min.

ΔFeed; Water; ∇Product; Water; ●Feed; Water switched to synthetic apple juice at 120 min ■Product; water switched to synthetic apple juice at 120 min.

In Fig. 6, we processed synthetic apple juice and observed approximately 1.5 log kill of bacteria at 40C in the microwave unit. At 3 h, we increased the cooling water flow rate to lower the temperature of the synthetic apple juice in the microwave chamber to 23C. At 23C there was no effect indicating that temperature is important. We think the microwave energy imposes a thermal effect which accelerates the natural killing effect of malic acid on *Pediococcus* sp.

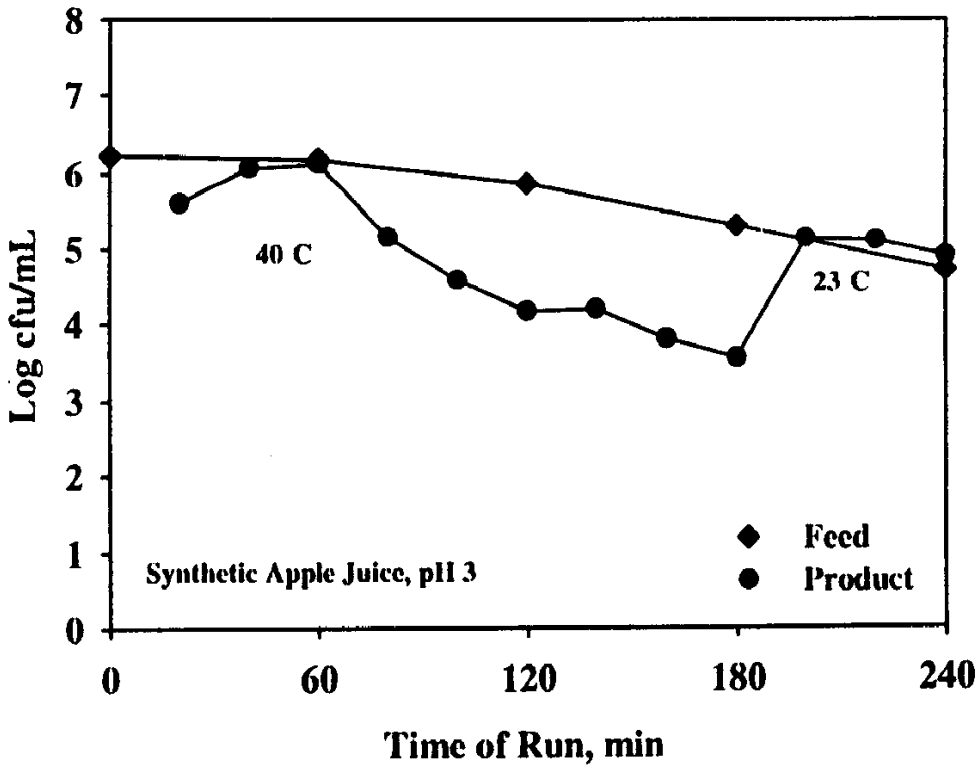


FIG. 6. EFFECT OF TEMPERATURE ON THE SUBLETHAL MICROWAVE PROCESS III RESULTS WITH *PEDIOCOCCUS* SP. IN SYNTHETIC APPLE JUICE (pH 3)
◆Feed; ●Product. Microwave unit turned on at 60 min; cooling water increased at 180 min.

The process was also tested on a nonpathogenic bacteria, *E. coli* in apple juice, grown in TGY. The bacteria was unaffected in the microwave chamber, indicating that malic acid has no effect on the *E. coli*. It also indicates that we were unable to detect a nonthermal effect associated with microwave energy.

## CONCLUSIONS

The process is capable of applying microwave energy to a continuous steady state process while simultaneously removing the thermal energy to maintain a low temperature. The process runs at turbulent flow to attain a uniform temperature and time distribution. There is no convincing evidence that microwave energy can kill microorganisms without thermal energy or other contributing factors. Microwave energy, in the absence of other stresses such as heat, pH, or antimicrobials, did not kill microorganisms at low temperatures in our continuous process. Although refuting the concept of low temperature destruction of microorganisms, there is still the possibility that microwave energy may complement or magnify thermal effects.

## REFERENCES

ANNOUS, B.A. and KOZEMPEL, M.F. 1998. Influence of growth medium on thermal resistance of *Pediococcus* sp. NRRL B-2354 (formerly Micrococcus freudenreichii) in liquid foods. J. Food Protect. *61*(5), 578-581.

ANON. 1985. Deactivation of microorganisms by an oscillating magnetic field. World patent 85/02094. Assigned to Maxwell Laboratories, Inc., San Diego, California.

BROWN, G.H. and MORRISON, W.C. 1953. Exploration of the effects of strong radiofrequency fields on micro-organisms in aqueous solutions. Food Technol. *8*(8), 361-366.

CARROLL, D.E. and LOPEZ, V. 1969. Lethality of radio frequency energy upon microorganisms in liquid, buffered, and alcoholic food systems. J. Food Sci. *34*, 320-324.

CULKIN, K.A. and FUNG, D.Y.C. 1975. Destruction of *Escherichia coli*, and *Salmonella typhimurium* in microwave-cooked soups. J. Milk Food Technol. *38*(1), 8-15.

DATTA, A.K. and LIU, J. 1992. Thermal time distributions for microwave and conventional heating of food. Trans IchemE *70*(C), 83-90.

GOLDBLITH, S.A. 1966. Basic principles of microwaves and recent developments. Adv. Food Res. *15*, 273-301.

GOLDBLITH, S.A. and WANG, D.I.C. 1967. Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. Applied Microbiol. 1371-1375.

KINOSITA, K., JR. and TSONG, T.Y. 1977. Nature *268*, 438-441.

KNORR, D., GEULEN, M., GRAHL, T. and SITZMANN, W. 1994. Food application of high electric field pulses. Trends Food Sci. Technol. *5*, 71-75.

KOZEMPEL, M., ANNOUS, B., COOK, R., SCULLEN, O.J. and WHITING, R. 1998. Inactivation of bacteria with microwaves at reduced temperatures. J. Food Protec. *61*(15), 582-585.

KOZEMPEL, M., SCULLEN, O.J., COOK, R. and WHITING, R. 1997. Preliminary investigation using a batch flow process to determine bacteria destruction by microwave energy at low temperatures. Lebensmittel-Wissenchaft und -Technologie *30*(7), 691 -696.

MERTENS, B. and KNORR, D. 1992. Developments of non-thermal processes for food preservation. Food Technol. *46*(5), 124-133.

NELSON, S.O. and CHARITY, L.F. 1972. Frequency dependence of energy absorption by insects and grain in electric fields. Trans. ASAE *15*(6), 1099-1102.

OLSEN, C. M. 1965. Microwaves inhibit bread mold. Food Eng. *37*(7), 51-53.

RAMASWAMY, H.S. and TAJCHAKAVIT, S. 1993. Continuous-flow microwave heating of orange juice. Paper No. 933588 at the ASAE Meeting.

REZNIK, D. and KNIPPER, A. 1994. Method of electroheating liquid egg and product thereof. U.S. patent 5,290,583.

TONG, C.H. 1996. Effect of microwaves on biological and chemical systems. Microwave World *17*(2), 14-23.

WEBB, S.J. and DODDS, D.D. 1968. Inhibition of bacterial cell growth by 136 gc microwaves. Nature *218*, 374-375.

WEBB, S.J. and DODDS, D.D. 1969. Absorption of microwaves by microorganisms. Nature *222*, 1199-1120.

ZIMMERMANN, U., PILWAT, G. and RIEMANN, F. 1974. Dielectric breakdown at cell membranes. Biophys. J. *14*, 881-889.